PHYSIOLOGY IN MEDICINE

In collaboration with
The American Physiological Society, Thomas E. Andreoli, MD, Editor

Apoptosis in Rheumatic Diseases

Tamara Grodzicky, MD, Keith B. Elkon, MD

Apoptosis, or programmed cell death (PCD), is a form of cellular demise that occurs when cells are damaged or no longer needed. It plays an important role in embryogenesis, normal tissue homeostasis, as well as in certain pathologic conditions, such as in oncogenesis, acquired immunodeficiency syndrome (AIDS), certain neurodegenerative diseases, and in autoimmunity (1). The term "apoptosis" was coined in 1972 by Kerr and colleagues (2), and further developments in the field, particularly in the last decade, paralleled advances in molecular biology, genetics, and biochemistry.

GENERAL MECHANISMS OF APOPTOSIS AND SIGNAL TRANSDUCTION

Although apoptosis is a complex, regulated process, the basic program has been highly conserved during evolution. The genetic control of apoptosis was first established in the nematode *Caenorhabditis elegans* and found to comprise four relatively distinct phases: initiation, effector, degradation, and receptor-mediated engulfment of apoptotic bodies by phagocytes (3). The various biochemical, molecular, and genetic factors modulating apoptosis in mammalian cells are much more complex than in the nematode.

Ultimately, the fate of the cell depends on the balance between pro-apoptotic and anti-apoptotic stimuli. The gene products of current interest involved in regulating PCD belong to two broad categories: proto-oncogenes, the most important ones belonging to the Bcl-2 family, some of which are anchored to mitochondria, and tumor-suppressor genes, such as p53.

Aside from these genetic influences, the cell is also subjected to many other apoptosis modifiers, such as various cytokines (eg, IL-4, IL-2, IL-10), as well as circulating or membrane-bound molecules capable of triggering specific ligand-receptor interactions. The prototypical death receptors are Fas (CD95; APO-1) and tumor necrosis factor receptor I and II (TNF-RI and TNF-RII), and their corresponding ligands are FasL and TNF-α, respectively (4) (Figure 1).

Signaling by Fas

Fas is expressed on many different cell types, whereas FasL is mainly expressed on activated Th1 T cells. FasL induces trimerization of the receptor and clustering of its intracellular death domain (4). This leads to recruitment of the cytoplasmic protein Fas-associated death domain (FADD), which subsequently triggers the proteolytic cascade of caspases that ultimately leads to cell death. Each step is regulated.

The major role of the Fas pathway of PCD is the termination of immune responses by causing peripheral deletion of activated mature T and B lymphocytes. However, it is also involved in preventing inflammation in "immune-privileged" sites, such as the eye and testis, where FasL is constitutively expressed. Another important function of this pathway is the killing of virus-infected or transformed cells (5). Mutations in Fas or FasL are associated with peripheral lymphoid expansion and autoimmune disease (6–8).

Signaling by TNF-RI

TNF-α is a soluble cytokine produced by activated T lymphocytes and macrophages in response to inflammation and infection. After binding to the TNF-RI, receptor clustering occurs, with recruitment of the adaptor protein TNF receptor–associated death domain (TRADD). In most cells, TNF-R engagement leads to activation of the transcription factors NF-kB and AP-1, leading to cell activation and proliferation. However, apoptosis can occur depending on the cell type, the receptor (TNF-RI or TNF-RII) engaged, and on the interplay of other regulators. Although Fas and TNF receptors are the best characterized, a number of other death receptors have recently been identified, such as death receptor 3 (DR3; also called APO-3/TRAMP/WSL-1/LARD), death receptor 4 (DR4), and 5 (DR5; also called APO-2/TRAIL-R2/TRICK 2/KILLER). The roles of these new death pathways in immune regulation and in human diseases remain to be determined (9).

Ligand–death receptor interactions lead to triggering of an intracellular proteolytic cascade involving "caspases"

Am J Med. 2000;108:73–82.
From the Department of Medicine, Rheumatology Division, Hospital for Special Surgery, Weill Medical College of Cornell University, New York, New York.

Requests for reprints should be addressed to Keith B. Elkon, MD, Research Division, Hospital for Special Surgery, Weill Medical College of Cornell University, 535 East 70th Street, New York, New York 10021.

0002-9343/00/$–see front matter
PII S0002-9343(00)00332-0

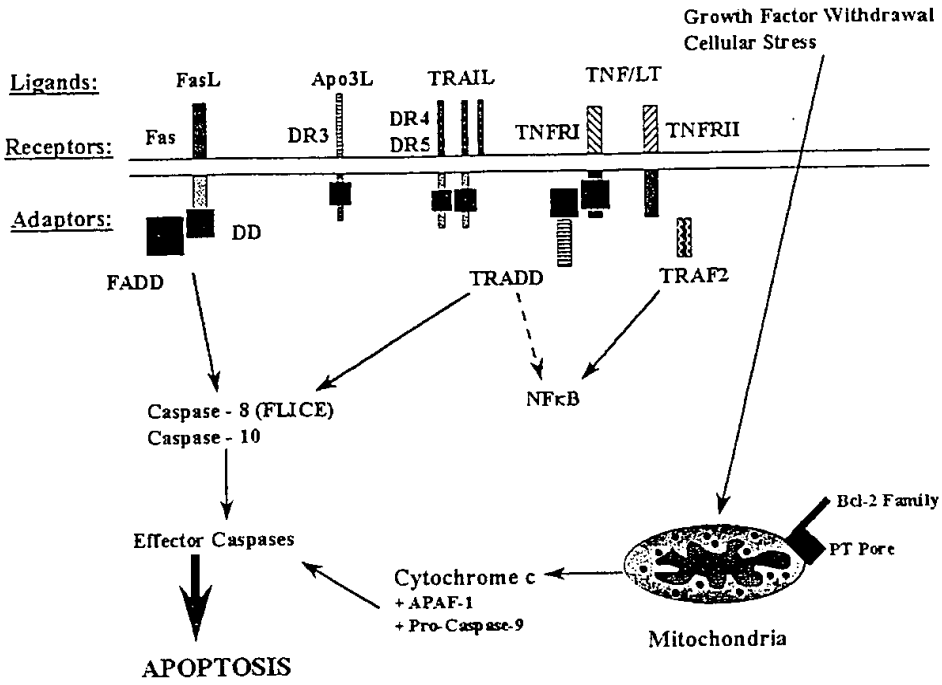

Figure 1. Cell death pathways. Inducers and early signal transduction pathways of the major known apoptotic pathways. Interaction of the ligand with its receptor causes receptor clustering and recruitment of the adaptor molecules. These, in turn, facilitate enzymatic activity of the caspases. Note that tumor necrosis factor α (TNF-α) may induce a death signal through a TNF receptor I (TNF-RI)–associated death domain (TRADD) or stimulate cell activation through the nuclear factor κB (NF-κB) pathway. Growth factor withdrawal or stress leads to the release of cytochrome c from the mitochondria. Cytochrome c, apoptotic protease activating factor-1 (APAF-1), and ATP activate pro-caspase-9, leading to the activation of downstream effector caspases (see text). The Bcl-2 family of proteins participate in this regulation. DD = death domain; DED = death effector domain; FADD = Fas-associated death domain; FasL = Fas ligand; FLICE = FADD-like interleukin-1β-converting enzyme; LT = lymphotoxin; PT pore = permeability transition pore; TRAF2 = TNFR2-associated factor 2.

(cysteine aspartate proteases). In the case of most death receptors, caspases 8 or 10 are activated. Cell death may also be initiated by an "intrinsic pathway," for example, after growth factor withdrawal. In this case, release of cytochrome c from mitochondria together with such cofactors as Apaf-1 (apoptotic protease activating factor-1) and ATP lead to caspase 9 activation (Figure 1). Similarly to the clotting and complement cascades, the caspase cascade proceeds in an autocatalytic manner, leading to amplification of the initial apoptotic signal. The cascade is regulated at the posttranslational level, by protein–protein interactions (10). Another group of proteases involved in PCD is the family of serine proteases, the most important member being Granzyme B. The Granzyme B–perforin pathway is a critical component of granule exocytosis and the main apoptotic mechanism involved in target-cell PCD induced by cytotoxic T cells and natural killer cells (11).

ROLE OF APOPTOSIS IN RHEUMATIC DISEASES

Apoptosis in Autoimmune Diseases

The fundamental function of the immune system is to protect the individual from infectious organisms (nonself). Lymphocytes play a key role in this process and are equipped with unique receptors to monitor the antigens to which the host is exposed. To be able to recognize the universe of foreign antigens, T and B lymphocyte antigen receptors are randomly generated. The consequence of this strategy is that lymphocytes with reactivity against self antigens are also produced and, to allow the host to survive, must be eliminated or held in check.

The concept of immunologic tolerance was evoked to explain the failure of cells of the immune system to react against self antigens. Although tolerance is usually described in terms of the self–nonself dichotomy, lymphocytes cannot distinguish self from nonself. Rather, the immune system has evolved numerous strategies to ensure that an immune response against self antigens does not occur. Some of these strategies occur in the central lymphoid organs [eg, deletion of high-affinity (self) reactive lymphocytes in the thymus (T cells) and bone marrow (B cells)], whereas others are achieved in the peripheral immune system (spleen, lymph nodes, and lymphoid tissue of the mucosa).

The Fas/FasL pathway of PCD plays an important role in peripheral tolerance, by halting the unwanted expansion of activated T- and B-cell clones beyond the course

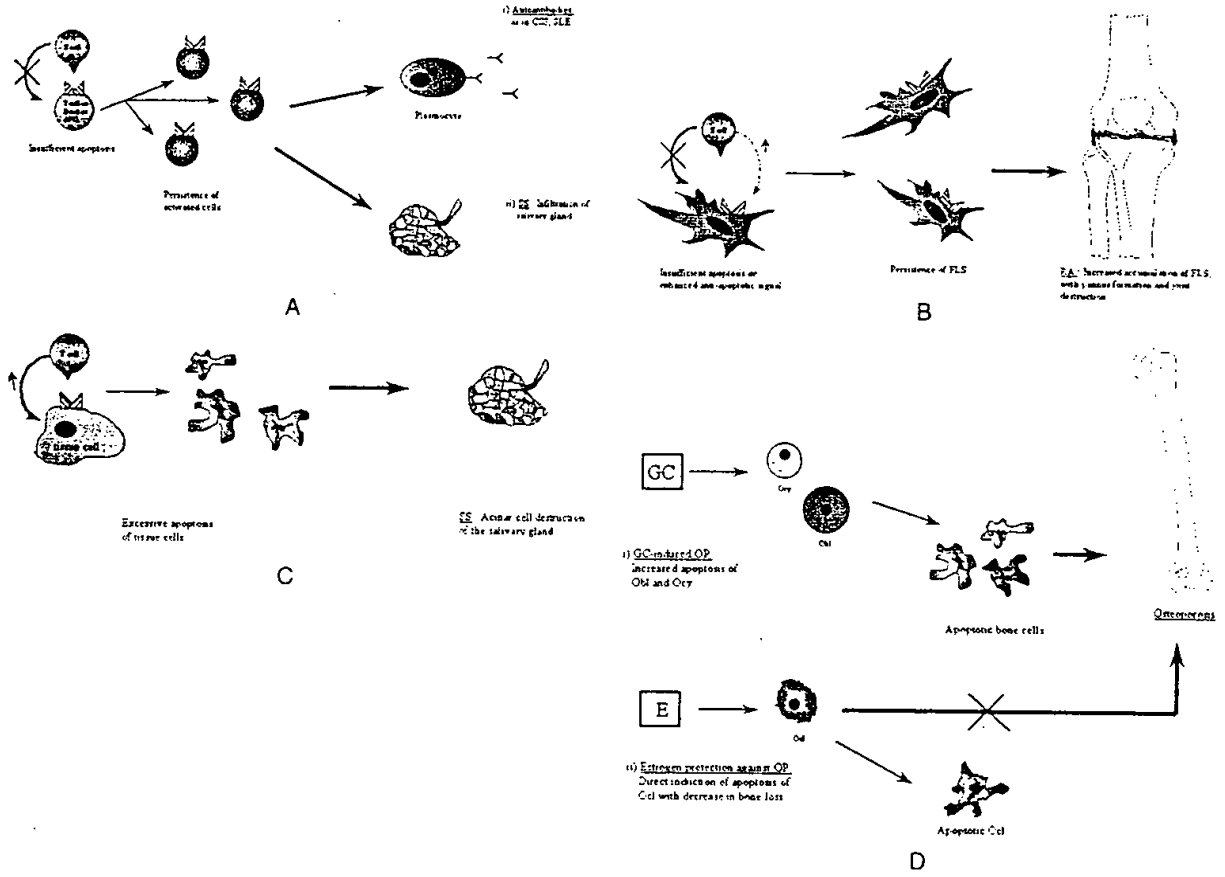

Figure 2. Schematic representation of the role of defective apoptosis in the physiopathology of several rheumatic diseases. (A) Insufficient apoptosis (✗) of T or B lymphocytes and possibly of other antigen-presenting cells (APC), such as macrophages or dendritic cells, after the binding of the death ligand (▼) to its receptor (≧), leading to lymphoaccumulation and i) enhanced B-cell accumulation with antibody production, as seen in systemic lupus erythematosus (SLE)–like diseases, such as the Canale-Smith Syndrome (CSS). ii) Abnormal lymphocytic infiltration of salivary glands in Sjögren's Syndrome (SS) with resulting glandular enlargement. (B) Insufficient apoptosis (✗) of fibroblast-like synoviocytes (FLS), or perhaps an increased anti-apoptotic signal (⚡) from T cells, in the pathophysiology of rheumatoid arthritis (RA). The net result is persistence and increased proliferation of FLS, pannus formation, and joint destruction. (C) Excessive apoptosis (↯) of tissue cells by adjacent activated T cells, with subsequent acinar cell destruction in SS salivary glands. (D) Excessive apoptosis of bone cells, a possible contribution to the pathogenesis of osteoporosis (OP). i) Glucocorticoids (GC) enhance apoptosis of osteoblasts (Obl) and osteocytes (Ocy), the cells responsible for bone formation, thereby inducing OP. ii) Estrogen (E) can directly cause programmed cell death in osteoclasts (Ocl), the major bone-resorbing cells, thereby protecting against OP. Estrogen withdrawal or deficiency may therefore contribute to bone loss by decreased Ocl demise (see text).

of an infection, and by eliminating clones reacting with self-antigen (4). Some systemic autoimmune disorders are a consequence of defective apoptosis of activated lymphocytes (Figure 2A). This was first discovered in mice that have mutations of Fas (*lpr*) or FasL (*gld*) and develop a lupus-like autoimmune disease with autoantibodies, glomerulonephritis, and a generalized lymphadenopathy. A similar disease, but with less severe features of autoimmunity, has been described in humans with Fas mutations [Canale-Smith Syndrome (8), autoimmune lymphoproliferative syndrome (7), human lymphoproliferative syndrome (6)]. Defects in other genes involved in the regulation of apoptosis have been shown to produce lupus-like syndromes in mice. Examples include Bcl-2 overexpression (12), defective expression of IL-2 or the α and β chains of its receptor (13), and defective B-cell antigen receptor signal transduction (14).

Systemic lupus erythematosus. Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by multiple organ involvement and the presence of a wide variety of serum autoantibodies (15). At present, it is unclear whether defects in apoptosis pathways contribute to either defective tolerance or antigen selection in this disorder. In contrast to the mice with lupus and lymphoproliferation, most patients with SLE do not have reduced Fas expression or function (16,17). However, the fact that rare cases of SLE are associated

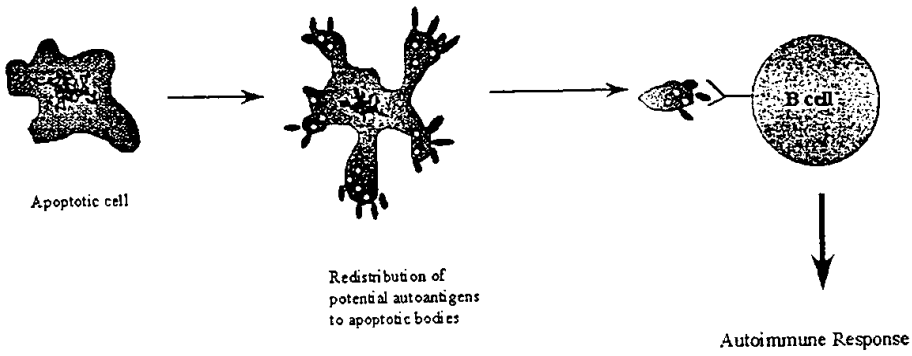

Figure 3. Apoptosis as a source of immunogens. During early programmed cell death, there is externalization of phosphatidylserine (o o) and redistribution of various nuclear antigens (a), such as Ro, La, and nucleosomal DNA, to the surface of the cell within apoptotic bodies. Increased apoptosis or abnormal processing may directly trigger B cells to initiate an autoimmune response (see text).

with either Fas (18) or FasL mutations (19) suggests that defective function of a related death effector could play a role in the pathogenesis of SLE. Levels of soluble Fas receptor (sFas), a secreted form of Fas that is able to inhibit FasL binding in vitro, is present in the plasma of many patients with SLE. However, this finding is not specific to SLE, and the precise role of sFas in disease pathogenesis has yet to be clarified (20,21).

Other regulators of PCD may be abnormal in human lupus. Although increased Bcl-2 expression leads to lupus-like disease in mice, data concerning human SLE are controversial. A recent genetic study reported a synergistic effect in disease susceptibility between Bcl-2 and IL-10 alleles in Mexican-American patients with SLE (22).

Defective apoptosis may contribute to SLE-like diseases in another way. Ultraviolet exposure of keratinocytes causes a redistribution of many nuclear antigens (eg, Ro, La, and nucleosomal DNA) to near the surface of the cell in apoptotic bodies (23,24). Furthermore, apoptosis is associated with the externalization of phosphatidylserine, which may be important in the generation of antiphospholipid antibodies (25). Because lymphocytes from lupus patients have an activated phenotype and undergo accelerated PCD compared with normal individuals in vitro, it has been suggested that apoptotic bodies contain modified self-antigens that could be immunogenic (Figure 3). To test experimentally whether apoptotic cells could induce an autoimmune response, Mevorach et al (26) immunized mice with large amounts of apoptotic cells. They observed that these cells induce a transient immune response (antiphospholipid and anti-ss-DNA autoantibodies). These experimental data support the indirect evidence that autoantibodies in SLE target the products of apoptotic cells, but further research is needed.

Rheumatoid arthritis. Rheumatoid arthritis (RA) is a systemic autoimmune disease associated with high morbidity and increased mortality. The hallmark of RA is synovial inflammation and proliferation, ultimately leading to joint destruction. At the cellular level, there is considerable hyperplasia of synoviocytes, mainly the "fibroblast-like synoviocytes" (FSL) cells, located in the deeper layer of the synovium, which acquire invasive potential (27). With the ingress of various inflammatory cells into the sublining area, the final result is synovial thickening, with pannus formation and bony erosions. A number of pro-inflammatory cytokines are expressed in the inflamed synovium, IL-1, IL-6, and TNF-α.

Regulation of apoptosis of several cell types is highly relevant to the pathogenesis of RA. Fas and FasL are constitutively expressed in the RA synovium. Both are detected on FLS and mononuclear cells, but the death-inducing FasL is mainly expressed on CD4+ (helper) and CD8+ (cytotoxic) lymphocytes invading the synovium. FLS of RA are sensitive to anti-Fas antibody, in contrast to osteoarthritis (OA) synoviocytes expressing similar levels of Fas (28). Thus, PCD-mediated elimination of Fas+ synoviocytes and mononuclear cells should theoretically occur by the FasL-expressing lymphocytes (Figure 2B). However, only a small proportion of these cells (less than 1%) actually undergo spontaneous apoptosis (29). Such a low apoptotic rate in the Fas + cells of the rheumatoid synovium may be explained by a relative deficiency of FasL (30), the presence of high amounts of soluble FasL in the synovial fluid, competing with its membrane-bound counterpart and thereby blocking Fas-mediated apoptosis (FMA) (31), or may be an artefact related to very rapid uptake of apoptotic cells by adjacent macrophages (3,32).

Recent evidence from studies on murine models of inflammatory arthritis indicates that enhancing FMA may have therapeutic applications. The intra-articular administration of anti-Fas monoclonal antibody to transgenic mice resulted in marked and rapid clinical improvement of joint inflammation, with significant increase of apo-

ptosis of synovial fibroblasts and mononuclear cells (33). Similarly, the intraperitoneal injection of anti-Fas into mice with severe combined immune deficiency (SCID; lacking mature T and B lymphocytes) with engrafted human RA synovial tissue was equally beneficial, although more toxic (34,35). Intra-articular FasL gene transfer increased the rate of apoptosis in synovial fibroblasts by cell-to-cell interaction via the Fas/FasL (36,37).

The high proliferative rate of the synovium and its ability to invade and destroy cartilage and bone have led several authors to compare it with tumors (27). This "transformed" phenotype may be explained by the altered expression or function of anti-apoptotic genes, oncogenes, or tumor suppressors. Among the anti-apoptotic genes, Bcl-xL, survivin (38), and Bcl-2 were reported to be overexpressed in RA FLS and T cells (39,40), possibly contributing to synovial hyperplasia. Defects in B-cell apoptosis in RA have also been proposed to explain the local and especially the systemic autoimmunity observed (41). Somatic mutations in the p53 gene have also been reported in RA synoviocytes and mononuclear cells, but not in RA skin, nor in normal controls or OA synovium (42). These mutations occur in "hotspots," the same regions of the gene mutated in human neoplasias, suggesting that they may have functional importance. The majority of p53 mutations seen in RA synovium are characteristic of oxidative deamination by nitric oxide (NO), suggesting that the NO generation in rheumatoid joints resulting from chronic inflammation may contribute to these somatic genetic modifications (43). Furthermore, NO has been recently implicated in playing a role in the prevention of Fas-mediated apoptosis, by its ability to S-nitrosylate and thus prevent activation of caspases (44). Other genes may also be altered in RA, such as H-ras (45).

Sjögren's syndrome. Sjögren's syndrome is an autoimmune disease characterized by destruction of the salivary and lacrimal glands associated with a mononuclear cell infiltration and, in some cases, with lymphoproliferation. The disease may occur in an isolated form (primary) or be associated with other connective tissue diseases, such as RA or SLE (secondary). The destruction of the exocrine glands are, in part, mediated by apoptosis of the acinar and ductal epithelial cells where caspase activation has been demonstrated in the acinar-epithelial cells (46) (Figure 2C). Salivary glands constitutively express Fas but not FasL on acinar epithelium. It has been suggested that gland destruction results from cytokine-induced expression of FasL and/or overexpression of Bax in salivary acinar cells (47). Expression of Fas in normal glands has, however, not been consistently observed. No mutations in Fas or FasL have been found in these patients to date (48–50).

Lymphocytic infiltrates in this disease are, most likely, caused by enhanced attraction by pro-inflammatory chemokines and cytokines and by deficient PCD (Figure 2A). Bcl-2 expression has been shown to be increased in the mononuclear cells infiltrating the salivary glands of patients with primary Sjögren's syndrome and this correlated with resistance to PCD in vitro (47). These findings could explain lymphoproliferation and the potential for B-cell "pseudolymphomas" that develop in a proportion of patients with long-standing disease.

Several mouse models of Sjögren's syndrome exist. These include lupus-prone strains, NZB and MRL/*lpr*, and a diabetes-prone strain, the nonobese diabetic (NOD) mouse (51). NOD mice constitutively express FasL in their exocrine glands, and Fas was detected on their salivary epithelium, suggesting a role for FMA in the observed glandular destruction. Lymphocytes of NOD mice are resistant to various apoptosis-inducing signals, possibly explained by enhanced expression of the anti-apoptotic molecule Bcl-xL in CD4+ T cells (52). Intriguingly, NOD-*scid* mice (lacking mature, functional T and B lymphocytes) develop apoptosis of submandibular acinar cells, suggesting that apoptosis of acinar epithelial cells may reflect an intrinsic defect within the glands and that lymphocytic infiltration is a secondary phenomenon (46,53). Deficient expression of certain lymphocyte costimulatory molecules may also contribute to the observed apoptotic resistance of invading immune cells (54).

Systemic sclerosis. Systemic sclerosis is a disease characterized by progressive fibrosis of the skin and also internal organs, such as the lung, kidney, gastrointestinal tract, and heart. Although the late fibrotic stage of the disease is associated with the accumulation of collagenous and noncollagenous extracellular matrix, the early lesions exhibit prominent perivascular and tissue infiltration by mononuclear inflammatory cells. The exact cause of this disease remains obscure, but the possibility of apoptosis as an initiating and/or perpetuating event has been suggested by several authors. Studies of early lesions suggest that apoptosis of endothelial cells, possibly as a result of cytotoxic anti-endothelial cell antibodies, may occur before the infiltration of lymphocytes (55,56). The finding that Bcl-2 is upregulated in cultured dermal fibroblasts from patients with systemic sclerosis (57) could be relevant to the progressive fibrosis that occurs later in this disease if these cells can be shown to be resistant to apoptosis.

Myopathies. Inflammatory myopathies, such as polymyositis and dermatomyositis, are autoimmune diseases that result in destruction of skeletal muscle fibers. Although Fas is upregulated on the myocytes in these diseases, expression is also increased in nonautoimmune muscle disorders, such as in metabolic myopathies, denervating disorders, and muscular dystrophies, but not in normal human muscle tissue. Detection of FasL on

mononuclear cells invading the muscles in patients with polymyositis and dermatomyositis with apoptosis of muscle cells is of greater pathogenetic relevance and implicates Fas/FasL in tissue injury in myositis (58). However, because high expression of the T-cell cytotoxic mediator, perforin, was also found in some patients with polymyositis and dermamyositis (59), other mechanisms of myocyte injury may also be involved.

Antiphospholipid syndrome. Antiphospholipid antibodies occur in approximately a third of SLE patients and are associated with arterial and venous thrombosis, thrombocytopenia, and first-trimester abortion. A subset of these antibodies bind to either a complex formed between anionic phospholipids and the plasma protein beta2-glycoprotein I (beta2GPI), or against beta2GPI alone. During PCD, phosphatidylserine is flipped from the inner to outer surface of the cell membrane, rendering it potentially accessible to the immune system (25). This early apoptotic event may lead to the generation of an autoimmune response (Figure 3). In a recent study, the injection of mice with beta2GPI and apoptotic cells induced antibodies harboring antiphospholipid antibodies and lupus anticoagulant activities (60). Binding of beta2GPI to apoptotic cells may facilitate their clearance by phagocytes (61–63). In addition to their putative role as immunogens, certain antiphospholipid antibodies may induce PCD in endothelial cells (64), thereby rendering the vascular endothelium procoagulant (65).

Apoptosis in Other Rheumatic Diseases

Osteoarthritis. The main mechanism underlying either primary or secondary OA is the degradation of cartilage. Such degradation is mediated by enzymatic and NO-induced breakdown of the extracellular matrix with insufficient new matrix synthesis. Apoptosis of chondrocytes occurs during normal skeletal development, and several groups have reported that it could also be important in experimental and in human OA. Normal human chondrocytes of the superficial and middle zones of cartilage, the major areas involved in early cartilage degeneration, express Fas and are somewhat sensitive to Fas-mediated killing (66). Human OA chondrocytes have also been shown to express Fas but have enhanced spontaneous apoptosis in the corresponding zones compared with normal controls (67,68). These OA chondrocytes were also shown to be sensitive to anti-Fas antibody. Although NO is also capable of inducing PCD in chondrocytes, it does not seem to interact with the Fas pathway (66). Interestingly, a recent study showed that transgenic mice lacking type II collagen, the main constituent of the extracellular matrix in cartilage, had high levels of apoptosis in their chondrocytes (69). Although further research is necessary, these findings suggest that PCD may play a role in OA and that inhibitors of NO synthesis may be of value in treating this disease. The therapeutic use of intra-articular anti-Fas antibody, although possibly useful to decrease RA synovial inflammation, may potentially be deleterious to chondrocytes.

Osteoporosis. Osteoporosis (OP) is a common disorder resulting from either increased bone resorption, decreased bone synthesis, or a combination of both. An increased rate in apoptosis of osteoblasts and osteocytes, the major bone-forming cells, has been reported in glucocorticoid-induced OP (70) (Figure 2D). Estrogen may exert its beneficial effect in preventing OP by its ability to directly induce apoptosis in the bone-resorbing osteoclasts (71) (Figure 2D), in a caspase-dependent fashion (72).

In addition to numerous other modulating factors influencing bone-turnover cell survival, such as cytokines (73), a novel protein, osteoprotegerin/osteoclastogenesis-inhibitory factor, has recently been shown to inhibit osteoclasts after binding to its cognate ligand (osteoprotegerin ligand/TRANCE) (74). As with other members of this TNF-R family, osteoprotegerin also has immunomodulatory properties, such as promotion of dendritic cell survival (74,75).

Seronegative spondyloarthropathies. Psoriasis and psoriatic arthritis are conditions that are associated with excessive cellular proliferation of the epithelium and of the synovium, respectively, possibly resulting from defective apoptotic pathways in these tissues. Keratinocytes, synovial cells, and dermal and synovium-derived fibroblast cell lines from patients with psoriatic arthritis overexpress p53 compared with normal and OA control specimens (76). As in RA, it remains to be determined whether p53 plays a direct role in the pathogenesis of disease.

APOPTOSIS REGULATION BY ANTI-INFLAMMATORY AND ANTIRHEUMATIC DRUGS

Therapy of rheumatic disorders is, at present, empiric. The types of drugs used include anti-inflammatory agents such as corticosteroids and nonsteroidals, immunomodulatory drugs such as cyclosporine, and cytotoxic drugs such as cyclophosphamide and azathioprine. Because most of these drugs impinge on critical biochemical events within the cell, they have important effects on apoptosis pathways.

Aspirin and Nonsteroidal Anti-inflammatory Drugs

Aspirin (acetylsalicylic acid [ASA]) and nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently consumed medications in the United States. The major mechanism of action of aspirin and NSAIDs is inhibition of cyclooxygenases (COXs), which in turn re-

duce the production of pro-inflammatory cytokines and prostaglandins (77). These drugs have also been shown to be effective in the chemoprevention of colorectal tumors in genetically susceptible individuals (78) and in rodents. A potential mechanism of their antineoplastic properties is their ability to induce apoptosis, probably via a COX-independent pathway (79,80). It has been suggested that NSAIDs' inhibition of COX activity and resulting increase of the prostaglandin precursor, arachidonic acid, promotes the conversion of sphingomyelin to ceramide, a potent pro-apoptotic lipid (79).

Glucocorticoids

Glucocorticoids (GCs) were liberally used for the treatment of autoimmune inflammatory disorders because of their potent anti-inflammatory and immunosuppressive action. GC have a wide range of biochemical effects including modulation of gene expression, induction of immune cell apoptosis (T and B cells, neutrophils), and regulation of various transcription factors, such as the impairment of NF-kB activation. They influence the expression of regulatory proteins, such as those involved in cell cycle control, c-myc, Bcl-2, p53, and certain cytokines (IL-2 and its receptor chain α, IL-6) (81).

Patients on long-term GC therapy are susceptible to OP and osteonecrosis, which may be explained by bone loss resulting from apoptosis of osteoblasts and osteocytes (70) (Figure 2D). GCs downregulate death-promoting proteases, such as the caspases (82), and Granzyme B (83), but act independently of the JNK/SAPK and Fas pathways. In in vitro studies, GC-suppressed apoptosis of Fas-expressing osteoblasts by soluble or membrane-bound FasL derived from activated human PBMCs (84), which could in part explain the recent observation that low-dose GC administration may exert a protective effect against bone erosions and periarticular osteopenia in inflammatory arthritides, such as RA.

Methotrexate

Methotrexate is a folic-acid antagonist that inhibits DNA synthesis at high doses and can cause apoptosis of cancer cells in vitro. However, whether such PCD is Fas dependent remains controversial. In rheumatic diseases, much lower doses are used such that minimal toxicity is observed. One mode of action of low-dose methotrexate is increased adenosine release at sites of inflammation, which suppresses neutrophil-mediated tissue injury by means of adenosine A2 receptors (85). In addition, several recent studies have reported that low-dose methotrexate induces apoptosis of activated lymphocytes in vitro and in patients with RA, probably in a Fas-independent manner (86,87). Methotrexate may also exert its beneficial effect on psoriatic skin disease by induction of PCD of keratinocytes (88).

TNF Blockade

TNF, the ligand for TNF-RI and TNF-RII, is a pro-inflammatory cytokine that has pleiomorphic effects depending on the cell type and environment. TNF-α plays an important and complex role in RA pathogenesis, by inducing proliferation of synoviocytes and angiogenesis. The administration of TNF-blocking agents, such as anti-TNF-α monoclonal antibodies (89) or soluble human recombinant TNF receptors (90,91), results in significant improvement of disease activity as a result, in part, of blockade of the pro-inflammatory effects of this cytokine and, possibly, as a result of increasing susceptibility to Fas (92).

Cyclosporine/FK506

FK506 (Tacrolimus) is a macrolide antibiotic with immunosuppressive properties, closely related to cyclosporine A (CsA). These two agents have been extensively used in the prevention of allograft rejection in transplant recipients and more recently have gained use in the treatment of RA. Both drugs modulate T- and B-cell immune responses by interfering with IL-2 gene transcription, NO synthase activation, cell degranulation, and apoptosis (93). Their immunosuppressive potential is achieved, in part, by increased apoptosis of autoreactive T and B lymphocytes, and may similarly affect PCD of cells involved in synovial inflammation (94). CsA nephrotoxicity may also be explained by increased apoptotic cell death of renal proximal tubules, possibly by means of the Fas/FasL pathway (95,96).

Paradoxically, CsA and FK 506 are also noted to have anti-apoptotic properties, possibly by effecting down-regulation of FasL expression on cytotoxic T lymphocytes. This may account for their capacity to inhibit the cytotoxic T lymphocyte–mediated killing of organ allografts (97–99). Apart from their therapeutic usefulness in immune modulation, they have also been shown to inhibit apoptosis of neuronal and endothelial cells, and may therefore prove to be useful in halting the progression of certain neurodegenerative and vascular diseases (100,101).

Cyclophosphamide

Cyclophosphamide is an alkylating agent commonly used to treat many human cancers and severe autoimmune disease. Its efficacy has partly been attributed to PCD-induced destruction of tumor cells and perhaps mesangial cells in glomerulonephritis (102). Induction of apoptosis may also account for certain adverse effects, such as oligo- or azoospermia (103), pancreatic β-cell destruction (104), and teratogenesis (105,106). Apoptosis is mostly effected through the p53-dependent pathway.

Bisphosphonates

Bisphosphonates are the most potent antiresorptive drugs available and are widely used to treat various metabolic bone diseases, such as Paget's disease, bone tu-

mors, ectopic calcifications, and OP. Although each member of this family has its own physicochemical and biologic properties and potencies, the following mechanisms of action have been reported: direct suppression of osteoclast activity, direct and indirect inhibition of osteoclast recruitment, and osteoclast apoptosis (107,108). The molecular pathways leading to PCD are still unclear but are possibly mediated by caspase activation (109) and by the modulation of certain GTP-binding proteins, such as Ras (110). Moreover, it has been suggested that bisphosphonates may have anti-inflammatory potential by their ability to cause apoptosis in macrophages (cells ontogenetically related to osteoclasts) in certain rat models (111).

CONCLUSIONS

This review summarizes the current knowledge about the role of apoptosis in the pathophysiology underlying several important rheumatologic conditions, and its role in the modulation of disease activity by some of the major therapeutic agents used to treat them. The intense ongoing research in this field will better define the different pathways and molecules that influence the pathogenesis of these diseases. This information will also aid in the design of specific therapeutic strategies, ultimately leading to more efficient disease control with minimal toxicity to the patient.

REFERENCES

1. Strasser A, Huang DC, Vaux DL. The role of the bcl-2/ced-9 gene family in cancer and general implications of defects in cell death control for tumourigenesis and resistance to chemotherapy. *Biochim Biophys Acta.* 1997;1333:F151–F178.
2. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer.* 1972;26:239–257.
3. Savill J, Fadok V, Henson P, Haslett C. Phagocyte recognition of cells undergoing apoptosis. *Immunol Today.* 1993;14:131–136.
4. Nagata S, Golstein P. The Fas death factor. *Science.* 1995;267: 1449–1456.
5. Nagata S. Apoptosis by death factor. *Cell.* 1997;88:355–365.
6. Rieux LF, Le Deist F, Hivroz C, et al. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science.* 1995;268:1347–1349.
7. Fisher GH, Rosenberg FJ, Straus SE, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell.* 1995;81:935–946.
8. Drappa J, Vaishnaw AK, Sullivan KE, et al. Fas gene mutations in the Canale-Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. *NEJM.* 1996;335:1643–1649.
9. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science.* 1998;281:1305–1308.
10. Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science.* 1998;281:1312–1316.
11. Greenberg AH. Activation of apoptosis pathways by Granzyme B. *Cell Death Differ.* 1996;3:269–274.
12. Strasser A, Whittingham S, Vaux DL, et al. Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc Natl Acad Sci USA.* 1991;88:8661–8665.
13. Suzuki H, Kündig TM, Furlonger C, et al. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science.* 1995;268:1472–1476.
14. Hibbs ML, Tarlinton DM, Armes J, et al. Multiple defects in the immune system of Lyn-deficient mice, culminating in autoimmune disease. *Cell.* 1995;83:301–311.
15. Elkon KB. Autoantibodies in systemic lupus erythematosus. *Curr Opin Rheumatol.* 1995;7:384–388.
16. Mysler E, Bini P, Drappa J, et al. The apoptosis-1/Fas protein in human systemic lupus erythematosus. *J Clin Invest.* 1994;93: 1029–1034.
17. Ohsako S, Hara M, Harigai M, et al. Expression and function of Fas antigen and bcl-2 in human systemic lupus erythematosus lymphocytes. *Clin Immunol Immunopathol.* 1994;73:109–114.
18. Vaishnaw AK, Orlinick JR, Chu JL, et al. The molecular basis for apoptotic defects in patients with CD95 (Fas/Apo-1) mutations. *J Clin Invest.* 1999;103:355–363.
19. Wu J, Wilson J, He J, et al. Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. *J Clin Invest.* 1996;98:1107–1113.
20. Cheng J, Zhou T, Liu C, et al. Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. *Science.* 1994;263: 1759–1762.
21. Jodo S, Kobayashi S, Kayagaki N, et al. Serum levels of soluble Fas/APO-1 (CD95) and its molecular structure in patients with systemic lupus erythematosus (SLE) and other autoimmune diseases. *Clin Exp Immunol.* 1997;107:89–95.
22. Mehrian R, Quismorio FPJ, Strassmann G, et al. Synergistic effect between IL-10 and bcl-2 genotypes in determining susceptibility to systemic lupus erythematosus. *Arthritis Rheum.* 1998;41:596–602.
23. Golan TD, Elkon KB, Gharavi AE, Krueger JG. Enhanced membrane binding of autoantibodies to cultured keratinocytes of systemic lupus erythematosus patients after ultraviolet B/ultraviolet A irradiation. *J Clin Invest.* 1992;90:1067–1076.
24. Casciola RL, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J Exp Med.* 1994;179:1317–1330.
25. Martin SJ, Reutelingsperger CP, McGahon AJ, et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J Exp Med.* 1995;182:1545–1556.
26. Mevorach D, Zhou JL, Song X, Elkon KB. Systemic exposure to irradiated apoptotic cells induces autoantibody production. *J Exp Med.* 1998;188:387–392.
27. Lafyatis R, Remmers EF, Roberts AB, et al. Anchorage-independent growth of synoviocytes from arthritic and normal joints. Stimulation by exogenous platelet-derived growth factor and inhibition by transforming growth factor-beta and retinoids. *J Clin Invest.* 1989;83:1267–1276.
28. Firestein GS, Yeo M, Zvaifler NJ. Apoptosis in rheumatoid arthritis synovium. *J Clin Invest.* 1995;96:1631–1638.
29. Matsumoto S, Muller-Ladner U, Gay RE, et al. Ultrastructural demonstration of apoptosis, Fas and Bcl-2 expression of rheumatoid synovial fibroblasts. *J Rheumatol.* 1996;23:1345–1352.
30. Cantwell MJ, Hua T, Zvaifler NJ, Kipps TJ. Deficient Fas ligand expression by synovial lymphocytes from patients with rheumatoid arthritis. *Arthritis Rheum.* 1997;40:1644–1652.
31. Hashimoto H, Tanaka M, Suda T, et al. Soluble Fas ligand in the

joints of patients with rheumatoid arthritis and osteoarthritis. *Arthritis Rheum.* 1998;41:657–662.
32. Cohen JJ. Apoptosis. *Immunol Today.* 1993;14:126–130.
33. Fujisawa K, Asahara H, Okamoto K, et al. Therapeutic effect of the anti-Fas antibody on arthritis in HTLV-1 tax transgenic mice. *J Clin Invest.* 1996;98:271–278.
34. Sakai K, Matsuno H, Morita I, et al. Potential withdrawal of rheumatoid synovium by the induction of apoptosis using a novel in vivo model of rheumatoid arthritis. *Arthritis Rheum.* 1998;41: 1251–1257.
35. Ogasawara J, Watanabe-Fukunaga R, Adachi M, et al. Lethal effect of the anti-Fas antibody in mice. *Nature.* 1993;364:806–809.
36. Zhang H, Yang Y, Horton JL, et al. Amelioration of collagen-induced arthritis by CD95 (Apo-1/Fas)-ligand gene transfer. *J Clin Invest.* 1997;100:1951–1957.
37. Okamoto K, Asahara H, Kobayashi T, et al. Induction of apoptosis in the rheumatoid synovium by Fas ligand gene transfer. *Gene Ther.* 1998;5:331–338.
38. Pap T, Franz JK, Kuchen S, et al. Expression of Survivin, a novel anti-apoptotic molecule, in the synovium of patients with rheumatoid arthritis (RA). *Arthritis Rheum.* 1998;41:S275. Abstract.
39. Sioud M, Mellbye O, Forre O. Analysis of the NF-kappa B p65 subunit, Fas antigen, Fas ligand and Bcl-2-related proteins in the synovium of RA and polyarticular JRA. *Clin Exp Rheumatol.* 1998; 16:125–134.
40. Petrow PK, Carsiens I, Gaumann A, et al. Expression of apoptosis-related molecules in the synovial membrane of patients with rheumatoid arthritis. *Arthritis Rheum.* 1998;41:S276. Abstract.
41. Shimaoka Y, Attrep JF, Hirano T, et al. Nurse-like cells from bone marrow and synovium of patients with rheumatoid arthritis promote survival and enhance function of human B cells. *J Clin Invest.* 1998;102:606–618.
42. Firestein GS, Echeverri F, Yeo M, et al. Somatic mutations in the p53 tumor suppressor gene in rheumatoid arthritis synovium. *Proc Natl Acad Sci USA.* 1997;94:10895–10900.
43. Harris CC. 1995 Deichmann Lecture—p53 tumor suppressor gene: at the crossroads of molecular carcinogenesis, molecular epidemiology and cancer risk assessment. *Toxicol Lett.* 1995;82–83: 1–7.
44. Mannick JB, Hausladen A, Liu L, et al. Fas-induced caspase denitrosylation. *Science.* 1999;284:651–654.
45. Roivainen A, Jalava J, Pirila L, et al. H-ras oncogene point mutations in arthritic synovium. *Arthritis Rheum.* 1997;40:1636–1643.
46. Robinson CP, Yamachika S, Alford CE, et al. Elevated levels of cysteine protease activity in saliva and salivary glands of the non-obese diabetic (NOD) mouse model for Sjögren syndrome. *Proc Natl Acad Sci USA.* 1997;94:5767–5771.
47. Kong L, Ogawa N, McGuff HS, et al. Bcl-2 family expression in salivary glands from patients with primary Sjögren's syndrome: involvement of Bax in salivary gland destruction. *Clin Immunol Immunopathol.* 1998;88:133–141.
48. Kong L, Ogawa N, Nakabayashi T, et al. Fas and Fas ligand expression in the salivary glands of patients with primary Sjögren's syndrome. *Arthritis Rheum.* 1997;40:87–97.
49. Kong L, Robinson CP, Peck AB, et al. Inappropriate apoptosis of salivary and lacrimal gland epithelium of immunodeficient NOD-scid mice. *Clin Exp Rheumatol.* 1998;16:675–681.
50. Elkon KB. Fas (APO-1/CD95)-assisted suicide in NOD exocrine glands. *Clin Exp Rheumatol.* 1998;16:659–661.
51. Kikutani H, Makino S. The murine autoimmune diabetes model: NOD and related strains. *Adv Immunol.* 1992;51:285–322.
52. Lamhamedi CS, Luan JJ, Eloy L, et al. Resistance of T-cells to apoptosis in autoimmune diabetic (NOD) mice is increased early in life and is associated with dysregulated expression of Bcl-x. *Diabetologia.* 1998;41:178–184.
53. Kong LO, Robinson CP, Peck AB, et al. Inappropriate apoptosis of salivary and lacrimal gland epithelium of immunodeficient NOD-scid mice. *Clin Exp Rheumatol.* 1998;16:675–681.
54. Colucci F, Bergman ML, Penha G, et al. Apoptosis resistance of nonobese diabetic peripheral lymphocytes linked to the Idd5 diabetes susceptibility region. *Proc Natl Acad Sci USA.* 1997;94:8670–8674.
55. Sgonc R, Gruschwitz MS, Dietrich H, et al. Endothelial cell apoptosis is a primary pathogenetic event underlying skin lesions in avian and human scleroderma. *J Clin Invest.* 1996;98:785–792.
56. Bordron A, Dueymes M, Levy Y, et al. The binding of some human antiendothelial cell antibodies induces endothelial cell apoptosis. *J Clin Invest.* 1998;101:2029–2035.
57. Pablos JL, Santiago B, Carreira PE, et al. Bcl-2 is upregulated in resting scleroderma fibroblasts. *Arthritis Rheum.* 1998;41:S321. Abstract.
58. Sugiura T, Murakawa Y, Nagai A, et al. Fas and Fas ligand interaction induces apoptosis in inflammatory myopathies: CD4+ T cells injury in polymyositis. *Arthritis Rheum.* 1999;42:291–298.
59. Goebels N, Michaelis D, Engelhardt M, et al. Differential expression of perforin in muscle-infiltrating T cells in polymyositis and dermatomyositis. *J Clin Invest.* 1996;97:2905–2910.
60. Levine JS, Subang R, Koh JS, Rauch J. Induction of anti-phospholipid autoantibodies by beta2-glycoprotein I bound to apoptotic thymocytes. *J Autoimmun.* 1998;11:413–424.
61. Manfredi AA, Rovere P, Galati G, et al. Apoptotic cell clearance in systemic lupus erythematosus. I. Opsonization by antiphospholipid antibodies. *Arthritis Rheum.* 1998;41:205–214.
62. Manfredi AA, Rovere P, Heltai S, et al. Apoptotic cell clearance in systemic lupus erythematosus. II. Role of beta2-glycoprotein I. *Arthritis Rheum.* 1998;41:215–223.
63. Rovere P, Manfredi AA, Vallinoto C, et al. Dendritic cells preferentially internalize apoptotic cells opsonized by anti-beta2-glycoprotein I antibodies. *J Autoimmun.* 1998;11:403–411.
64. Nakamura N, Ban T, Yamaji K, et al. Localization of the apoptosis-inducing activity of lupus anticoagulant in an annexin V-binding antibody subset. *J Clin Invest.* 1998;101:1951–1959.
65. Bombeli T, Karsan A, Tait JF, Harlan JM. Apoptotic vascular endothelial cells become procoagulant. *Blood.* 1997;89:2429–2442.
66. Hashimoto S, Setareh M, Ochs RL, Lotz M. Fas/Fas ligand expression and induction of apoptosis in chondrocytes. *Arthritis Rheum.* 1997;40:1749–1755.
67. Hashimoto S, Ochs RL, Komiya S, Lotz M. Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. *Arthritis Rheum.* 1998;41:1632–1638.
68. Blanco FJ, Guitian R, Vázquez ME, et al. Osteoarthritis chondrocytes die by apoptosis. A possible pathway for osteoarthritis pathology. *Arthritis Rheum.* 1998;41:284–289.
69. Yang C, Li SW, Helminen HJ, et al. Apoptosis of chondrocytes in transgenic mice lacking collagen II. *Exp Cell Res.* 1997;235:370–373.
70. Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC. Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. *J Clin Invest.* 1998;102:274–282.
71. Kameda T, Mano H, Yuasa T, et al. Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. *J Exp Med.* 1997;186:489–495.
72. Okahashi N, Koide M, Jimi E, et al. Caspases (interleukin-1beta-converting enzyme family proteases) are involved in the regulation of the survival of osteoclasts. *Bone.* 1998;23:33–41.
73. Jilka RL, Weinstein RS, Bellido T, et al. Osteoblast programmed cell death (apoptosis): modulation by growth factors and cytokines. *J Bone Miner Res.* 1998;13:793–802.
74. Yasuda H, Shima N, Nakagawa N, et al. Osteoclast differentiation

factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci USA.* 1998;95:3597–3602.
75. Kong YY, Yoshida H, Sarosi I, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature.* 1999;397:315–323.
76. Espinoza LR, van Solinger R, Cuellar ML, et al. p53 overexpression in psoriatic skin, synovium and fibroblasts. *Arthritis Rheum.* 1998; 41:S335. Abstract.
77. Vane JR, Botting RM. Mechanism of action of antiinflammatory drugs. *Int J Tissue React.* 1998;20:3–15.
78. Giardiello FM. NSAID-induced polyp regression in familial adenomatous polyposis patients. *Gastroenterol Clin North Am.* 1996; 25:349–362.
79. Chan TA, Morin PJ, Vogelstein B, Kinzler KW. Mechanisms underlying nonsteroidal antiinflammatory drug-mediated apoptosis. *Proc Natl Acad Sci USA.* 1998;95:681–686.
80. Schwenger P, Bellosta P, Vietor I, et al. Sodium salicylate induces apoptosis via p38 mitogen-activated protein kinase but inhibits tumor necrosis factor-induced c-Jun N-terminal kinase/stress-activated protein kinase activation. *Proc Natl Acad Sci USA.* 1997;94: 2869–2873.
81. Nocentini G, Giunchi L, Ronchetti S, et al. Glucocorticoids: regulation of gene expression and apoptosis. *J Chemother.* 1998;10: 187–191.
82. McColl KS, He H, Zhong H, et al. Apoptosis induction by the glucocorticoid hormone dexamethasone and the calcium-ATPase inhibitor thapsigargin involves Bcl-2 regulated caspase activation. *Mol Cell Endocrinol.* 1998;139:229–238.
83. Wargnier A, Lafaurie C, Legros-Maïda S, et al. Down-regulation of human granzyme B expression by glucocorticoids. Dexamethasone inhibits binding to the Ikaros and AP-1 regulatory elements of the granzyme B promoter. *J Biol Chem.* 1998;273:35326–35331.
84. Nakashima T, Sasaki H, Tsuboi M, et al. Inhibitory effect of glucocorticoid for osteoblast apoptosis induced by activated peripheral blood mononuclear cells. *Endocrinology.* 1998;139:2032–2040.
85. Cronstein BN. Molecular therapeutics. Methotrexate and its mechanism of action. *Arthritis Rheum.* 1996;39:1951–1960.
86. Paillot R, Genestier L, Fournel S, et al. Activation-dependent lymphocyte apoptosis induced by methotrexate. *Transplant. Proc.* 1998;30:2348–2350.
87. Genestier L, Paillot R, Fournel S, et al. Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. *J Clin Invest.* 1998;102:322–328.
88. Heenen M, Laporte M, Noel JC, de Graef C. Methotrexate induces apoptotic cell death in human keratinocytes. *Arch Dermatol Res.* 1998;290:240–245.
89. Elliott MJ, Maini RN, Feldmann M, et al. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum.* 1993;36:1681–1690.
90. Moreland LW, Baumgartner SW, Schiff MH, et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *NEJM.* 1997;337:141–147.
91. Weinblatt ME, Kremer JM, Bankhurst AD, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *NEJM.* 1999;340:253–259.
92. Kobayashi T, Okamoto K, Kobata T, et al. Tumor necrosis factor alpha regulation of the Fas-mediated apoptosis-signaling pathway in synovial cells. *Arthritis Rheum.* 1999;42:519–526.
93. Thomson AW, Bonham CA, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. *Ther Drug Monit.* 1995;17:584–591.
94. Cutolo M, Barone A, Accardo S, et al. Effect of cyclosporin on apoptosis in human cultured monocytic THP-1 cells and synovial macrophages. *Clin Exp Rheumatol.* 1998;16:417–422.
95. Thomas SE, Andoh TF, Pichler RH, et al. Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis. *Kidney Int.* 1998;53:897–908.
96. Healy E, Dempsey M, Lally C, Ryan MP. Apoptosis and necrosis: mechanisms of cell death induced by cyclosporine A in a renal proximal tubular cell line. *Kidney Int.* 1998;54:1955–1966.
97. Anel A, Buferne M, Boyer C, et al. T cell receptor-induced Fas ligand expression in cytotoxic T lymphocyte clones is blocked by protein tyrosine kinase inhibitors and cyclosporin A. *Eur J Immunol.* 1994;24:2469–2476.
98. Brunner T, Yoo NJ, LaFace D, et al. Activation-induced cell death in murine T cell hybridomas. Differential regulation of Fas (CD95) versus Fas ligand expression by cyclosporin A and FK506. *Int Immunol.* 1996;8:1017–1026.
99. Migita K, Eguchi K, Kawabe Y, et al. FK506 augments activation-induced programmed cell death of T lymphocytes in vivo. *J Clin Invest.* 1995;96:727–732.
100. Seaton TA, Cooper JM, Schapira AH. Cyclosporin inhibition of apoptosis induced by mitochondrial complex I toxins. *Brain Res.* 1998;809:12–17.
101. Walter DH, Haendeler J, Galle J, et al. Cyclosporin A inhibits apoptosis of human endothelial cells by preventing release of cytochrome C from mitochondria. *Circulation.* 1998;98:1153–1157.
102. Cha DR, Feld SM, Nast C, et al. Apoptosis in mesangial cells induced by ionizing radiation and cytotoxic drugs. *Kidney Int.* 1996; 50:1565–1571.
103. Cai L, Hales BF, Robaire B. Induction of apoptosis in the germ cells of adult male rats after exposure to cyclophosphamide. *Biol Reprod.* 1997;56:1490–1497.
104. Augstein P, Elefanty AG, Allison J, Harrison LC. Apoptosis and beta-cell destruction in pancreatic islets of NOD mice with spontaneous and cyclophosphamide-accelerated diabetes. *Diabetologia.* 1998;41:1381–1388.
105. Nomura M, Suzuki M, Suzuki Y, et al. Cyclophosphamide-induced apoptosis induces phocomelia in the mouse. *Arch Toxicol.* 1996;70:672–677.
106. Moallem SA, Hales BF. The role of p53 and cell death by apoptosis and necrosis in 4-hydroperoxycyclophosphamide-induced limb malformations. *Development.* 1998;125:3225–3234.
107. Fleisch H. Mechanisms of action of the bisphosphonates. *Medicina.* (Buenos Aires). 1997;57 (suppl 1):65–75.
108. Hughes DE, Wright KR, Uy HL, et al. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. *J Bone Miner Res.* 1995;10:1478–1487.
109. Coxon FP, Benford HL, Russell RG, Rogers MJ. Protein synthesis is required for caspase activation and induction of apoptosis by bisphosphonate drugs. *Mol Pharmacol.* 1998;54:631–638.
110. Luckman SP, Hughes DE, Coxon FP, et al. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *J Bone Miner Res.* 1998;13:581–589.
111. Rogers MJ, Chilton KM, Coxon FP, et al. Bisphosphonates induce apoptosis in mouse macrophage-like cells in vitro by a nitric oxide-independent mechanism. *J Bone Miner Res.* 1996;11:1482–1491.